Journal of Medical Virology 52:168–172 (1997)

Detection of Human Herpes Virus 8 DNA and Sequence Polymorphism in Classical, Epidemic, and Iatrogenic Kaposi's Sarcoma in South Africa

Susan Engelbrecht,[1]* Florette K. Treurnicht,[1] Johann W. Schneider,[2] H. Francois Jordaan,[3] Jan G. Steytler,[1] Peter A.B. Wranz,[2] and Estrelita J. van Rensburg[1]

[1]Department of Medical Virology University of Stellenbosch and Tygerberg Hospital, Tygerberg, South Africa
[2]Department of Anatomical Pathology University of Stellenbosch and Tygerberg Hospital, Tygerberg, South Africa
[3]Department of Dermatology University of Stellenbosch and Tygerberg Hospital, Tygerberg, South Africa

The aetiology and detection of human herpes virus type 8 (HHV-8) DNA sequences in Kaposi's sarcoma (KS) is a matter of intense investigation. We report on the detection of HHV-8 DNA and sequence polymorphism in different clinicopathological subtypes of cutaneous KS samples from South Africa. The diagnosis was confirmed by histological examination in all cases. Six patients had classic KS (CKS), 3 epidemic KS (EKS), and 3 iatrogenic KS (IKS). A nested polymerase chain reaction (PCR) assay was used to detect HHV-8 DNA in cell lysates, prepared from formalin fixed, paraffin embedded sections. We investigated polymorphism in the HHV-8 DNA using single-stranded conformational polymorphism (SSCP) analysis on the PCR products, followed by direct sequencing. HHV-8 DNA was detected in all the patients with KS, irrespective of the clinicopathological subtype. Direct sequencing was performed on 5 selected cases and showed single base pair substitutions in all. The spectrum of mutations was similar to those described previously. No correlation was found between the different types of KS and sequence variation. The results support the hypothesis that HHV-8 is strongly associated with different clinicopathological subtypes of KS and confirm the occurrence of HHV-8 in patients with CKS, EKS, and IKS in South Africa. ***J. Med. Virol. 52: 168–172, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: HHV-8; PCR; SSCP; mutations

INTRODUCTION

The cell of origin, aetiology, and therapy for Kaposi's sarcoma (KS) is under investigation [Schwarz, 1996]. KS is classified according to its different epidemiological manifestations. Classic KS (CKS) was originally described by Kaposi in 1872 as a rare cancer of elderly Mediterranean and Eastern European men. Endemic African KS (AKS) is the most common cancer among black adults in sub-Saharan Africa and is also seen in young children [Chang et al., 1996]. Caucasians from the southern African region where AKS is endemic present with CKS [Stein et al., 1994]. With the global human immunodeficiency virus (HIV) pandemic, KS has emerged as an unique complication of the acquired immune deficiency syndrome (AIDS), also named epidemic KS (EKS). Iatrogenic KS, including immunosuppressive treatment-related KS (IKS), was identified in transplant recipients. Despite the different epidemiological and clinical outcomes, all forms of KS have the same histology [Peterman et al., 1993] and may represent different manifestations of a single disease [De Lellis et al., 1995]. DNA sequences of a new human herpes virus, termed KS-associated herpes virus (KSHV), were identified recently in all forms of KS [Moore and Chang, 1995; Whitby et al., 1995]. The formal designation of this virus is human herpes virus type 8 (HHV-8) [Moore et al., 1996; Roizman, 1995].

In most patients with KS and AIDS, seroconversion to HHV-8 related latent nuclear antigens occur before the clinical appearance of KS [Gao et al., 1996]. HHV-8 sequences are present in KS lesions as large, covalently closed, circular episomes characteristic of latent herpes viruses, which were also detected in healthy donors [Decker et al., 1996]. The detection of RNA expression of HHV-8 in KS tumours further supports the possible etiologic and pathogenic role of this virus in the development of KS [Huang et al., 1996]. The virus is not

This work was performed at the Department of Medical Virology, University of Stellenbosch Medical School and Tygerberg Hospital.

*Correspondence to: Dr. Susan Engelbrecht, Department of Medical Virology, P.O. Box 19063, 7505 Tygerberg, South Africa. Email: se1@gerga.sun.ac.za.

Accepted 15 January 1997

restricted to the tumour tissue and may disseminate to many organs [Noel et al., 1996].

We report on the detection of HHV-8 DNA and sequence polymorphism in the various clinicopathological subtypes of KS in 12 South African patients with cutaneous KS.

MATERIALS AND METHODS

Patients and Samples

Biopsies of cutaneous KS from 12 patients—6 with CKS, 3 with EKS, and 3 with IKS (renal transplant patients)—were retrieved from the files of the Department of Anatomical Pathology, University of Stellenbosch and Tygerberg Hospital. Haematoxylin and eosin stained sections from formalin fixed, paraffin embedded tissues were examined histopathologically in all cases. Established criteria were used to classify the histopathological pattern in each case as early, spindle cell, or mixed pattern type [Harawi et al., 1989], corresponding respectively to patch, plaque, or nodular stages [Chor et al., 1992]. The clinical and demographic data for all patients were retrieved from the clinical files of the Department of Dermatology.

Preparation of Cell Lysates

Methods for the preparation of cell lysates were described previously [Van Rensburg et al., 1996]. To evaluate the possibility of contamination at this stage, 10 μm sections of a block containing normal fetal skin tissue were also included for evaluation. All sections were placed in separate Eppendorf Safe-Lock microfuge tubes (Eppendorf-Nethaler-Hinz GmbH, Hamburg, Germany) and stored at −20°C until use.

General procedures were used as recommended to avoid contamination during sample preparation [Kwok and Higuchi, 1989]. All samples were subjected to amplification using human β-globin primers to evaluate their suitability for DNA amplification [Saiki et al., 1986], before detection of HHV-8 DNA.

Nested PCR Conditions to Detect HHV-8 DNA

For HHV-8 PCR the outer primer set for the $KS330_{233}$ fragment and the internal probe used to detect the PCR product [Chang et al., 1994] were synthesised by Genosys Biotechnologies Inc. (The Woodlands, TX). Forty amplification cycles were used: denaturing of DNA (94°C; 1 minute), annealing of primers (58°C; 1 minute), and extension of the annealed primers (72°C; 1.5 minutes). PCR was performed using the following reagents: 10 μl of cell lysate, 200 μM of each nucleotide, 0.4 μM of each primer, 1 U of Taq DNA polymerase (Promega Corporation, Madison, WI), 10 μl of Promega 10x buffer, and 1.5 mM $MgCl_2$ made up in a final volume of 100 μl. Template DNA to amplify a 172 bp nested PCR product consisted of 10 μl of reaction product from the first round, using primers (5′-GTG CTC GAA TCC AAC GGA TT and 5′-ATG ACA CAT TGG TGG TAT AT) previously described [Whitby et al., 1995]. To standardise our HHV-8 PCR reactions, the $KS330_{233}$ plasmid control [Chang et al., 1995], was used to determine the number of target HHV-8 DNA molecules present as described previously [Engelbrecht and van Rensburg, 1995]. Termal cycling was carried out using a Perkin-Elmer GeneAmp® PCR system 9600 cycler (Perkin-Elmer Corporation, Norwalk, CT). Aliquots of 8 μl of the amplification reaction products were analysed by electrophoresis through 3% agarose gels stained with ethidium bromide and viewed by UV transillumination.

Detection of PCR Products by Probe Hybridisation

The DNA was denatured, and 4μl of the reaction product was spotted onto a nylon membrane (Amersham International, Buckinghamshire, England). The probes used to detect HHV-8 DNA were labeled using the DIG Oligonucleotide 3^1-end labeling kit (Boehringer Mannheim, Mannheim, Germany).

Hybridisation with the specific labeled probes was carried out overnight at 55°C in 6 × SSC, 5 × Denhardt's solution, 0.2% SDS and 100 μg/ml salmon sperm DNA. The filters were washed in 2 × SSC and 0.1% SDS for 5 minutes at 55°C, then 0.1 × SSC and 0.1% SDS at 55°C for 5 minutes, followed by 15 minutes at 55°C. The DNA hybridisation was detected using the DIG detection kit (Boehringer Mannheim, Mannheim, Germany).

Heteroduplex Single-Stranded Conformational Polymorphism (SSCP) Analysis

Polymorphism in the HHV-8 DNA samples was investigated using a combination of SSCP and direct sequencing. Heteroduplex-SSCP analysis of these fragments was done essentially as described, with a few modifications [Kotze et al., 1995]. Briefly, 10 μl of each PCR product was mixed with an equal volume of gel loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) containing 0.5 × TBE or 1.5 × TBE for either glycerol or ureum containing gels, denatured at 95°C for 2–5 minutes and immediately placed on ice. The samples were loaded on 24 cm vertical 1.5 mm thick (Hoefer Scientific Instruments, San Francisco, CA) 10% polyacrylamide gels with 1% cross-linking and run overnight at both room temperature and 12°C at 180 to 250 V (Bio-Rad Laboratories, Richmond, CA), depending on the size of the DNA fragments. Gels were supplemented with 7.5% and 5% urea and glycerol, respectively, stained for 10 minutes in a solution of 0.6 × TBE containing 1 μg/ml of ethidium bromide, and destained for 10 minutes in water before being evaluated by UV transillumination as described before.

Direct Sequencing

Sequencing of PCR products was done to confirm the presence of mutations that had been detected by heteroduplex-SSCP. PCR fragments from representative samples of EKS (number 9), CKS (numbers 2 and 5) and IKS (numbers 10 and 12) were sequenced. The primers used for sequencing were the same as those

TABLE I. Clinical and Histological Findings in 12 Patients With Kaposi's Sarcoma

Patient	Demographics age (years)/sex	KS type	HIV status	No. of lesions	Distribution	Morphology	Histology
1	82 M	CKS	Negative	Multiple	LL UL, trunk	Nodules	Mixed cell type (plaque)
2	76 F	CKS	Not done	Multiple	LL	Patches, papules, nodules	Spindle cell type (nodular)
3	49 M	CKS	Negative	Multiple	LL, UL, face	NR	Spindle cell type (nodular)
4	75 M	CKS	Not done	Multiple	LL, trunk	Nodules	Spindle cell type (nodular)
5	74 F	CKS	Not done	Solitary	LL	Nodule	Spindle cell type (nodular)
6	83 M	CKS	Not done	Multiple	LL	Papules	Spindle cell type (nodular)
7	40 M	EKS	Positive	Multiple	trunk	NR	Early type (patch)
8	46 M	EKS	Positive	Multiple	LL, UL, buttocks, trunk, scrotum	Papules	Mixed cell type (plaque)
9	42 F	EKS	Positive	Multiple	LL	Nodules, plaques	Mixed cell type (plaque)
10	50 F	IKS	Not done	Multiple	LL	Papules, plaques	Spindle cell type (nodular)
11	41 M	IKS	Not done	Multiple	LL	Papules	Mixed cell type (plaque)
12	44 M	IKS	Not done	Multiple	UL	Patches, papules, nodules	Early type (patch)

Abbreviations: M, Male; F, Female; UL, upper limb; LL, lower limb; NR, not recorded; CKS, classical KS; EKS, epidemic KS; IKS iatrogenic KS.

used for the nested PCR [Whitby et al., 1993]. The Sequenase PCR product sequencing kit (USB Catalogue US70170, supplied by Amersham Life Science) was used. Standard denaturing gel electrophoresis was done [Sambrook et al., 1989] using a glycerol tolerant gel buffer containing Taurine (Amersham International, Buckinghamshire, UK).

Sequence analysis

Sequences were visualised by autoradiography and read from both directions, and analysis was done with the Genepro V5.0 software program (Riverside Scientific Enterprises, Bainbridge Island, WA) by aligning it to the reference sequence of KS330 [Chang et al., 1994].

RESULTS

Clinical Features and Histopathologic Analysis

The clinical findings of the patients and histological features of the KS biopsies are summarised in Table I. The study group consisted of 12 patients, and the colour of the skin lesions were blue, red, purple and brown. Histopathologically, 6 of the 12 biopsies showed a spindle cell type, 4 cases a mixed cell type, and the remaining 2 cases early type Kaposi's sarcoma. Mitotic activity varied considerably but was absent in both of the early types cases (data not shown).

Five patients with CKS had multiple lesions on the lower limbs. The trunk (patients 1 and 4), upper limbs (patients 1 and 3), and face (patient 3) were also involved. Patient 5 displayed a solitary lesion on the dorsum of the right foot. Serology for HIV was negative in patients 1 and 3 and was not done in the remaining 4.

All 3 patients with EKS revealed multiple lesions with involvement of the trunk (patient 7), both limbs, the scrotum, and the buttocks (patient 8), and the lower limbs (patient 9). Two patients (patient, 10 and 11) with IKS had lesions on the lower limbs, and in patient 12, only the upper limbs were involved. HIV-serology of these 3 patients was not recorded.

TABLE II. HHV-8 Mutation Summary of South African KS

Patient no.	KS type	Nucleotide position	Nucleotide change	Amino acid change
2	CKS	1033	C → T	Pro → Leu
		1055	G → T	silent
		1132	A → G	Asp → Gly
		1139	A → C	silent
5	CKS	1086	C → T	silent
		1096	A → G	Tyr → Cys
		1139	A → C	silent
9	EKS	1132	A → G	Asp → Gly
		1139	A → C	silent
10	IKS	1086	C → T	silent
		1139	A → C	silent
12	IKS	1033	C → T	Pro → Leu
		1055	G → T	silent

PCR Amplification and Detection of HHV-8 PCR Products by Probe Hybridisation

All the samples were amplifiable with the β-globin primers (data not shown). Fetal skin samples placed between the study samples as well as the reagent controls were negative on PCR. A PCR sensitivity of 1 copy proviral genome could be detected with nested PCR.

HHV-8 DNA was detected in all samples. Five samples (patients 4, 6, 7, 8, and 11) were weakly positive on ethidium bromide stained gels but positive with hybridisation. Samples from patients 1 and 3 were negative on ethidium stained gels but weakly positive on hybridisation. The ethidium bromide stained gels and spot blot hybridisation reaction results are summarised in Figure 1.

SSCP Analysis and Direct Sequencing

Five of the 12 DNA fragments (patients 2, 5, 9, 10, and 12) showing an electrophoretic mobility shift were identified as positive for HHV-8 variation and were subsequently selected for DNA sequencing to confirm the SSCP results and to characterise the polymorphism. The weakly positive and negative samples seen on ethidium bromide stained gels after PCR were also

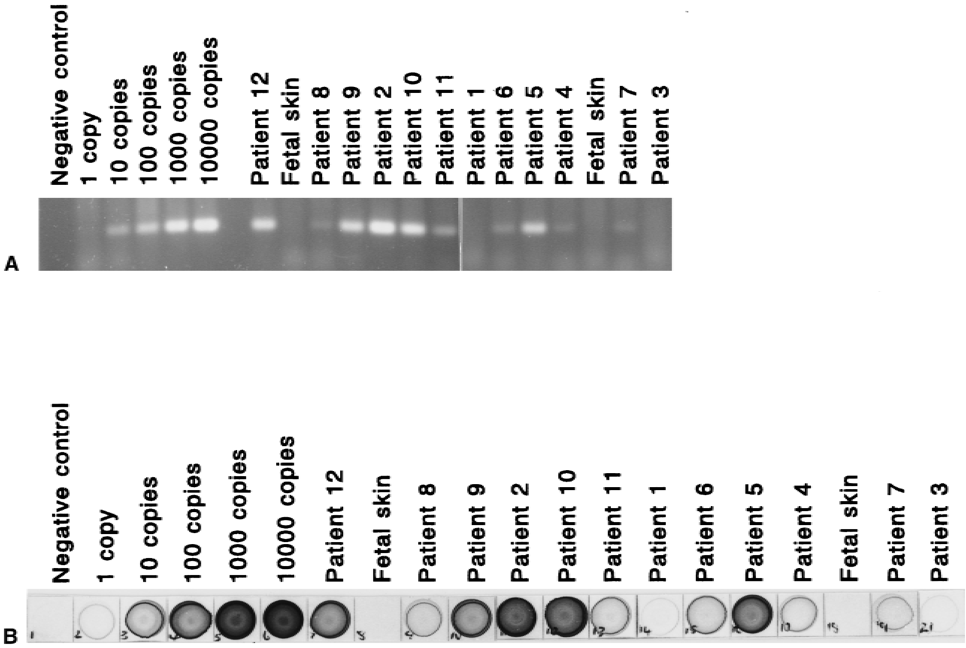

Fig. 1. PCR and dot blot analysis. **A:** An 8μl sample from each patient (as indicated above each lane) was run on 3% agarose gels and stained with ethidium bromide. The positive controls are represented as 1, 10, 100, 1000, and 10,000 copies. Negative controls are indicated. The fragment size is 172 bp. **B:** Dot blot analysis of PCR products presented in A.

weakly reactive with SSCP analysis and were not investigated further.

Sequences obtained from the 5 patients were aligned with the prototype sequence KS330. Mutations were detected in all the samples sequenced. In total, 6 point mutations were found, with 3 of them inducing an amino acid change (Table II). There were 4 transversions of which 2 were A-to-G changes (positions 1096 and 1132); one was a G-to-T change (position 1055) and one was an A-to-C change (position 1139). There were two C-to-T transitions (positions 1033 and 1086).

DISCUSSION

HHV-8 DNA has been demonstrated in KS in various epidemiological situations, in different population groups, and in a wide spectrum of histopathological patterns. The occurrence of HHV-8 DNA in KS in South Africa is unknown. The purpose of this study was to detect and determine the incidence of HHV-8 DNA present in cases of KS from our region. PCR detection of HHV-8 DNA is a specific and sensitive test to verify KS in the differential diagnosis of angioproliferative lesions [Dictor et al., 1996] and can be used to distinguish KS from other vascular lesions, particularly at its early stage [Jin et al., 1996]. In a study by Buonaguro et al. [1996], 100% of all types of KS, such as AKS, EKS, IKS, and CKS, tested positive for HHV-8 DNA by PCR and Southern blot. HHV-8 was identified in 85% of HIV-negative patients and in 92% of HIV-positive patients with KS in Uganda. Contrary to findings in studies in North America and Europe, 14% of Ugandan non-KS control patients' tissues were also positive for HHV-8 [Chang et al., 1996]. Endemic KS occurs frequently in Africa, and it is therefore likely that HHV-8 is more highly prevalent in Africa.

In Italy, one region where CKS is prevalent, HHV-8 sequences were identified in 2 of 5 (40%) patch, in 3 of 6 (50%) plaque, and in 10 of 11 (90.9%) nodular lesions [Luppi et al., 1996]. Noel et al., (1996) detected HHV-8 sequences in 58% of the early or patch stage and in 79% of the late plaque or nodular stages of KS.

In this study, HHV-8 sequences were detected in biopsies from all patients with different epidemiological variants of KS, namely, CKS, EKS, and IKS, as well as the various histopathological patterns of KS, including early type KS (patch stage) to mixed (plaque stage) and spindle cell types (nodular stage). Five of the 12 samples that were weakly positive and 2 that were negative on ethidium bromide stained gels were positive after spot blot hybridisation. Probe hybridisation enhanced the detection of amplified product and was necessary to confirm the specific amplification of HHV-8 DNA in all of the samples. Dictor et al. [1996] also found increased sensitivity using Southern blot hybridisation.

HHV-8 is prevalent in tissues from Kaposi's sarcoma and sequence analysis of amplification products revealed polymorphisms that result in amino acid changes of the predicted sequences [Collandre et al., 1995; Huang et al., 1995; Moore and Chang, 1995; O'Neill et al., 1996; Su et al., 1996]. Point mutations were also found in PCR products amplified from both prostate tissue and sperm samples [Monini et al., 1996]. The spectrum of mutations in our samples showed minor differences from the prototype sequence and was similar to those described previously (nucleo-

tide positions 1033, 1055, 1086, 1096, 1132, and 1039). In contrast to sequences from patients 9 (EKS) and 10 and 12 (IKS), where only 2 mutations were found, sequences from both patients with CKS showed 3 and 4 point mutations each, respectively. The significance of the sequence variation and the degree of divergence need to be investigated. No correlation between the different types of KS and sequence variation was found in our study.

To conclude, the results support the hypothesis that HHV-8 is strongly associated in all the clinicopathological types of KS and appears in patients with CKS, EKS and IKS in South Africa.

ACKNOWLEDGMENTS

This work was supported by grants from the Harry Crossley Foundation and the Cancer Association of South Africa. The authors thank Mr. D.H. Geiger for technical assistance.

REFERENCES

Buonaguro FM, Tornesello ML, Beth-Giraldo E, Hatzakis A, Mueller N, Downing R, Biryamwaho B, Sempala SD, Giraldo G (1996): Herpes virus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. International Journal of Cancer 65:25–28.

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS (1994): Identification of herpes virus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–1869.

Chang Y, Ziegler J, Wabinga H, Katangole-Mbidde E, Bosshoff C, Schultz T, Whitby D, Maddalena D, Jaffe HW, Weiss RA, Moore PS (1996): Kaposi's sarcoma-associated herpes virus and Kaposi's sarcoma in Africa. Archives Internal Medicine 156:202–204.

Chuck S, Grant RM, Katongole-Mbidde E, Conant M, Ganem D (1996): Frequent presence of a novel herpes virus genome in lesions of human immunodeficiency virus–negative Kaposi's sarcoma. Journal of Infectious Diseases 173:248–251.

Collandre H, Ferris S, Grau O, Montagnier L, Blanchard A (1995): Kaposi's sarcoma and new herpes virus. Lancet 345:1043.

Decker LL, Shanker P, Khan G, Freeman RB, Dezube BJ, Lieberman J, Thorley-Lawson DA (1996): The Kaposi sarcoma–associated herpes virus (KSHV) is present as an intact latent genome in KS tissue but replicates in the peripheral blood mononuclear cells of KS patients. Journal of Experimental Medicine 184:283–288.

De Lellis L, Fabris M, Cassai E, Corallini A, Giraldo G, Feo C, Monini P (1995): Herpes virus-like DNA sequences in non-AIDS Kaposi's sarcoma. Journal of Infectious Diseases 172:1605–1607.

Dictor M, Rambech E, Way D, Witte M, Bendsoe N (1996): Human herpes virus 8 (Kaposi's sarcoma–associated herpes virus) DNA in Kaposi's sarcoma lesions, AIDS Kaposi's sarcoma cell lines, endothelial Kaposi's sarcoma simulators, and the skin of immunosuppressed patients. American Journal of Pathology 148:2009–2016.

Engelbrecht S, van Rensburg EJ (1995): Detection of Southern African HIV-1 subtypes by PCR: Evaluation of different primer pairs and conditions. Journal of Virological Methods 55:391–400.

Gao SJ, Kingsley L, Hoover DR, Spira TJ, Rinaldo CR, Saah A, Phair J, Detels R, Parry P, Chang Y, Moore PS (1996): Seroconversion to antibodies against Kaposi's sarcoma-associated herpes virus related nuclear antigens before the development of Kaposi's sarcoma. New England Journal of Medicine 335:233–241.

Harawi SJ (1989): Kaposi's sarcoma. In Harawi SJ, O'Hara CJ (eds.): "Pathology and Pathophysiology of AIDS and HIV-Related Diseases." St. Louis, MO: C.V. Mosby, pp 83–131.

Huang YQ, Li JJ, Zhang WG, Feiner D, Friedman-Kien AE (1996): Transcription of human herpes virus–like agent (HHV-8) in Kaposi's sarcoma. Journal of Clinical Investigation 97:2803–2806.

Jin YT, Tsai ST, Yan JJ, Hsiao JH, Lee YY; Su IJ (1996): Detection of Kaposi's sarcoma-associated herpes virus-like DNA in vascular lesions. A reliable diagnostic marker for Kaposi's sarcoma. American Journal of Clinical Pathology 105:360–363.

Kotze MJ, Theart L, Callis M, Peeters AV, Thiart R and Langenhoven E (1995): Nonradioactive multiplex PCR screening strategy for the simultaneous detection of multiple low-density lipoprotein receptor gene mutations. PCR Methods and Application 4:352–356.

Kwok, S. and Higuchi, R. (1989) Avoiding false positives with PCR. Nature 339:237–238.

Luppi M, Barozzi P, Maiorana A, Collina G, Ferrari MG, Marasca R, Morselli M, Rossi E, Ceccherini-Nelli L, Torelli G (1996): Frequency and distribution of herpes virus–like DNA sequences (KSHV) in different stages of classic Kaposi's sarcoma and in normal tissues from an Italian population. International Journal of Cancer 66:427–431.

Mesri EA, Cesarman E, Arvanitakis L, Rafii S, Moore MA, Posnett DN, Knowles DM, Asch AS (1996): Human herpes virus-8/Kaposi's sarcoma-associated herpes virus is a new transmissible virus that infects B cells. Journal of Experimental Medicine 183:2385–2390.

Monini P, de Lellis L, Fabris M, Rigolin F, Cassai E (1996): Kaposi's sarcoma-associated herpes virus DNA sequences in prostate tissue and human semen. New England Journal of Medicine 334:1168–1172.

Moore PS, Chang Y (1995): Detection of herpes virus–like DNA sequences in Kaposi's sarcoma in patients with and in those without HIV infection. New England Journal of Medicine 332:1181–1185.

Moore PS, Gao S-J, Dominguez G, Cesarman E, Lungu O, Knowles DM, Garber R, Pellet PE, McGeoch DJ, Chang Y (1996): Primary characterisation of a herpes virus agent associated with Kaposi's sarcoma. Journal of Virology 70:549–558.

Noel J-C, Hermans P, Andre J, Fayt I, Simonart TH, Verhest A, Haot J, Burny A (1996): Herpes virus–like DNA sequences and Kaposi's sarcoma. Cancer 77:2132–2136.

O'Neill E, Henson TH, Ghorbani AJ, Land MA, Webber BL, Garcia JV (1996): Herpes virus-like sequences are specifically found in Kaposi's sarcoma lesions. Journal of Clinical Pathology 49:306–308.

Peterman TA, Jaffe HW, Beral V (1993): Epidemiologic clues to the etiology of Kaposi's sarcoma. AIDS 7:605–611.

Rady PL, Yen A, Rollefson JL, Orengo I, Bruce S, Hughes TK, Tyring SK (1995): Herpes virus–like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. Lancet 345:1339–1340.

Renne R, Zhong W, Herndier B, McGrath M, Abbey N, Kedes D, Ganem D (1996): Lytic growth of Kaposi's sarcoma–associated herpes virus (human herpes virus 8) in culture. Nature Medicine 2: 324–326.

Roizman B (1995): New viral footprints in Kaposi's sarcoma (editorial). New England Journal of Medicine 332:1227–1228.

Saiki RK, Bugawan TL, Horn GT, Mullis KB, Erlich HA (1986): Analysis of enzymatically amplified β-globin and HLA-DQα DNA with allele-specific oligonucleotide probes. Nature 324:163–166.

Sambrook J, Fritsch EF and Maniatis T, eds. (1989): Molecular cloning: A laboratory Manual, second edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Schwarz, RA (1996): Kaposi's sarcoma: Advances and perspectives. Journal of the American Academy of Dermatology 34:804–814.

Stein ME, Kantor A, Spencer D, Bezwoda WR (1994): Classical Kaposi's sarcoma in Caucasians in Africa–experience at the Johannesburg Hospital (1978–1992). Dermatology 188:182–187.

Su IJ, Huang LM, Wu SJ, Jin YT, Kao YF, Tsai TF, Lee JY, Hsu YH, Hsiao CH, Chang YC, Wang YW, Lee CY (1996): Detection and sequence analysis of a new herpes virus–like agent in AIDS and non-AIDS Kaposi's sarcoma in Taiwan. Journal of Formos Med Assoc (Taiwan) 95:13–18.

Tappero JW, Conant MA, Wolfe SF, Berger TG (1993): Kaposi's sarcoma. Epidemiology, pathogenesis, histology, clinical spectrum, staging criteria, and therapy. Journal of the American Academy of Dermatology 28:371–395.

Van Rensburg E, Engelbrecht S, van Heerden WFP, Raubenheimer EJ, Schoub BD (1996): Human papillomavirus DNA in oral squamous cell carcinomas from an African population sample. Anticancer Research 16:696–974.

Whitby D, Howard MR, Tenant-Flowers M, Brink NS, Copas A, Boshoff C, Hatzioannou T, Suggett FEA, Aldam DM, Denton AS, Miller RF, Weller IVD, Weiss RA, Tedder RS, Schulz TF (1995): Detection of Kaposi sarcoma associated herpes virus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. Lancet 346:799–802.